pathologic process that results from interruption of blood supply to the bone. The condition is extremely rare in healthy individuals, although it occurs usually before the fifth decade. The most common localization of idiopathic avascular necrosis is the femoral head, although it affects the knee as well. Pain often occurs only at an advanced stage of the condition.
Osteoarthritis (OA) conceived as a degenerative consequence of aging of the joint, with a well-characterized molecular pathophysiology, whereas rheumatoid arthritis (RA) is a common, inflammatory polyarthritis. The onset of RA varies from acute to insidious. The most common site of onset is in the hands and feet. Knee joints are also commonly affected, although it is not the initial joint.
Peripherially, or centrally released β-endorphin is an important indicator of pain and inflammation. As only a limited number of papers have been published before on the subject of the analysis of synovial fluid from hip and knee joint in different arthropathies, we wish to reply the question whether there is a difference in between the β-endorphin levels of patients with avascular necrosis, osetoarthritis and rheumatoid arthritis of the hip and knee. The role of β-endorphin in alleviation of pain has been well-described, while there are less data of its function in synovial fluid.
Methods: 87 patients (62 female, 25 male) were involved in our study with an average age of 62 (±11.27) years. 33 patients had avascular necrosis of stage IV, V, VI according to Steinberg's classification (18 hips, 15 knees). 23 patients suffered from OA (14 hips, 9 knees), whereas stage III-IV RA was diagnosed in 31 patients (12 hips, 19 knees) due to Steinbrocker's classification. Patients with OA and RA meet the ARA requirements. We measured the β-endorphin levels of the synovial fluids -harvested from surgery- with radioimmun assay (RIA).
Results: Our experiments showed elevated level of β-endorphin in synovial fluid of patients with AVN comparing to OA and RA, however significance was not proven due to a relatively high standard deviation. Nevertheless β-endorphin level was significantly higher in RA group than among patients with OA (p=0.012). Synovial β-endorphin level was measured lower in knee comparing to hip joint. When examining the different joints separately in compliance with diagnoses, we concluded that the synovial β-endorphin level from AVN was between the values of OA and RA without significance, whereas in RA it was significantly higher than from OA irrespectively of the joint (p=0.03 knee, p=0.013 hip).
Conclusions: synovial β-endorphin level in patients with inflammatory autoimmune diseases (e.g. RA), comparing to the level measured in degenerative conditions (e.g. OA). We interpret the higher β-endorphin level in AVN than in OA with the clinically well-known fact, that AVN is always accompanied by a synovial inflammation. The markedly higher β-endorphin level in patients with RA of the knee contrary to OA confirms the fact that the immune system has a strong impact on the expression of β-endorphin of opioid receprors and ligands of peripherial sensorial neurons.

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STERNAL FRACTURE AFTER MINOR TRAUMA IN A PATIENT WITH KYPHOSIS: A CASE REPORT

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Purpose: Older patients tend to suffer from osterarthritis of many joints and osteoporosis. In such cases, several types of fractures can easily occur. We present a case of sternal fracture after a subtle trauma in a patient with Kyphosis. 7 days after a very slight contact injury to her anterior chest, she suddenly experienced severe pain. To our knowledge, there are no reports of such a case.
Methods: We present a case of 69-year-old woman with kyposis. One day, her anterior chest was bumped slightly. She experienced mild discomfort in the anterior chest. She could, however, spend daily life without a difficulty. 7 days after, her discomfort then spontaneously transformed into severe pain and she visited a hospital, but the X-ray examination revealed no rib fractures. Nevertheless, severe pain persisted, so she visited our hospital 14 days after. On our first examination, her anterior chest pain was very severe. She could not move without help and a great deal of time was required to change her body position. In our first plain X-ray examination, we could confirm the step off fracture at the body of the sternum and many old vertebral compression fractures in her thoracic and lumbar spines. Fracture of the sternum was also confirmed by computed tomography, and there was no evidence of tumoral infiltration and no obvious abnormality in her mediastinum. Her bone density was examined using ultrasound, it was only 43% of the mean value for young adults. After confirming chest X-rays, electrocardiograms, Holter monitoring, echocardiograms, pulmonary function tests, laboratory tests failed to disclose the pathology, we made the diagnosis of sternal fracture secondary to severe osteoporosis and kyphosis.
Results: By conservative treatment with a rib band, 70 days after the onset, we could see the callus formation around the fracture and her discomfort became better. But, 3 months after, her discomfort became worse again and we confirm re-fracture at the same point. 6 months after, her pain disappeared and we could confirm callus on the X-ray. It took about a year to confirm sound bone union.
Conclusions: The sternum and ribs are usually protected from injury by the elasticity of the costal cartilage. However, these bones may become progressively ossified with age such that the deforming stress due to the thoracic kyphosis may be transmitted directly to the sternum (Sapherson, 1990). Thoracic kyphosis is thought to enhance the potential for a sternal insufficiency fracture by creating a deforming stress that exceeds the diminished elastic resistance of the osteoporotic bone (Cooper, 1988).
Based on the clinical course of our case, sternal insufficiency fracture should have been ruled out first. In our case, re-fracture was also seen at the same point during the treatment course. So, in the case of the sternal fracture patients with Kyphosis and osteoporosis, it might be better to take care of the possibility of re-fracture.
Conclusions: 1) In patients with osteoporosis and spinal compression fractures, sternal fracture must be considered if there are any complaints of discomfort in the anterior chest. 2) A lateral view radiograph of the sternum is important for the diagnosis of sternal fracture. 3) It might be better to take care of the possibility of re-fracture in the treatment of the sternal fracture patient with kyphosis and osteopolosis.

Pain: Pathophysiology

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VALIDATION OF CLINICAL PAIN ASSESSMENT METHODS WITH CANINE OSTEOARTHRITIS

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Purpose: The aim of this clinical study was to evaluate the construct and concurrent validation of chronic pain assessment methods in dogs diagnosed with osteoarthritis (OA). Different

behavioural and physiologic pain assessment methods were tested on OA dogs, and compared to peak vertical force (PVF) measured on gait analysis. Our hypothesis was that these instruments would correlate to PVF.

Methods: Twenty-three dogs with lameness were enrolled in the study. Lameness was confirmed with PVF gait analysis, radiographical and clinical orthopaedic diagnosis. Prior to the first evaluation, wash out periods were respected for OA pharmaceutical treatments, nutraceuticals, fatty acid supplement and therapeutic diets or threats. The clinical trial began with a 30-day period during which all dogs received a first diet. After this period, dogs received a second diet for a subsequent 60-days period. Owners evaluated pain using two behavioural rating scales: a standardized multifactorial questionnaire (MFQ) and a case-specific functional assessment (CODI) performed twice per week. Owners scored MFQ at D0, D30 and D90 with the assistance of a veterinarian. Clinical examination included measurement of electrodermal activity (EDA), body weight, and PVF at D0, D30 and D90. A pilot evaluation of locomotor activity including three parameters ["daily averaged total intensity" (DATI), "daily peak intensity" (DPI) and "daily proportion of active period" (DPAP)] was recorded every 2 minutes over the 90-day, using telemetric accelerometer (Actical®/Actiwatch®) on six dogs. Data analysis and statistics: PVF was corrected to body weight changes. MFQ and CODI were transformed to be ranged from 0 (higher pain) to 100 (lower pain).Outcomes of locomotor activity were averaged for every week of data acquisition. ANOVA for repeated measures followed by Tukey-Kramer multiple comparisons test were applied. Friedman test was used when justified. Spearman's rank correlation coefficient was fulfilled to evaluate construct validation.

Results: MFQ and CODI increased from D0 to D90 ($p<0.05$). EDA showed high variability and was unchanged over time. Body weight was changed over time, and PVF adjusted to body weight values were at D0: 65.7 (21.0), D30: 67.3 (19.7), and D90: 69.9 (21.3) ($p=0.03$). For locomotor activity, DATI and DPI were stable from D0 to D30 but increased from D0 to D90 ($p<0.05$).

CODI and MFQ were positively correlated (rho = 0.45, $p<0.001$). CODI was positively correlated to the DATI of locomotor activity over the 12 weeks of recording (rho = 0.42, $p<0.001$). PFV adjusted to body weight trended to correlate to DATI from D0 to D30 ($p=0.11$), and descriptive analysis demonstrated at D90 that PVF increase was also associated to increase in DATI, DPI and DPAP.

Conclusions: CODI, the behavioural pain rating scale developed for each specific dog and owner, was indicator of level of activity in dogs, but was not correlated to orthopaedic disability *per se*. Gait analysis was indicator of orthopaedic ability and endurance to be active. CODI and gait analysis had good construct validation and were complementary evaluations to evaluate chronic pain caused by OA in dogs. Locomotor activity looks as an objective behavioural evaluation and a potent complement to PVF gait analysis.

Proteomics & Metabolomics

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LEVELS OF SOD2 AND GRP78 ARE MODIFIED BY GLUCOSAMINE AND CHONDROITIN SULPHATE IN HUMAN ARTICULAR CHONDROCYTES: A PHARMACOPROTEOMIC STUDY

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Purpose: Glucosamine sulphate (GS) and chondroitin sulphate (CS) are symptomatic slow-acting drugs for osteoarthritis (OA) widely used in clinic. However, their mechanism of action remains poorly characterized. Since the variability in the treatment efficacy of these compounds is in accordance with the pathological OA degree (severe-moderate-mild), we decided to carry out our experiments in normal chondrocyte cultures stimulated with Interleukin-β. IL-1β is an arthritic mediator with the capacity to drive the key pathways typically associated with the pathogenesis of OA. The aim of this work is to explore the utility of a pharmacoproteomic approach for the identification of mechanisms and specific molecules involved in the pharmacological effect of glucosamine sulphate and chondroitin sulphate.

Methods: Chondrocytes obtained from 3 healthy donors were treated with GS 10mM and/or CS 200μg/mL, and then stimulated with IL-1β 10ng/mL. Whole cell proteins were isolated 24 hours after cellular stimulation and resolved by two-dimensional electrophoresis. The gels were stained with SYPRORuby and digitized using a CCD camera. The image analysis was performed using the PDQuest 7.3.1 computer software. Differentially abundant proteins were identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Visualization of modulated biological pathways was carried out using Pathway Studio software. Real-time and western blot analyses were performed to validate our results.

Results: We examined a mean of 500 protein spots that were present in each gel. Both qualitative and quantitative changes in protein expression patterns between controls and treated cells were studied. We identified 39 protein spots that were modulated by GS treatment, 35 by CS treatment and 48 by GS+CS treatment compared to the control. Database search showed that most of these proteins are involved in protein folding (PDIA1, PDIA3), stress response (HSP7C, HSPB1, ANXA2), cellular metabolism (AK1C2, PGK1, KPYM), protein targeting (GRP78) and oxidative stress (SOD2, PRDX1). Considering that oxidative stress balance has been reported to play an essential role in osteoarthritis, we found SOD2 upregulated by IL-1β and downregulated by GS and CS treatment, both at transcription and protein levels. Biological significance of these data included the drug-dependent decrease of superoxide dismutase activity that we detected in cartilage cells, and also the reduction in ROS generation. On the other hand, GRP78, a protein previously characterized by its anti-inflammatory properties and recently proposed as a potential new biologic therapy for RA, resulted increased by GS, alone and in combined administration. This is the first study carried out in chondrocytes that confirms the GS effect as positive modulator of Grp78 expression and points to a specific mechanism of action of this compound for its putative anti-inflammatory effect.

Conclusions: The present study uses an *in vitro* model of inflammation (using IL-1β) to describe the effect of GS and CS on cartilage cells. We have identified several novel molecular targets of these compounds, such as SOD2 and GRP78, which may